

SPERM STORAGE MECHANISMS AND FERTILIZATION IN FEMALES OF TWO SOUTH AMERICAN ELEDONIDS (CEPHALOPODA:OCTOPODA).

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ABSTRACT

Octopod species of the genus *Eledone* do not have spermathecae in the oviducal glands. Sperm masses are found within the ovary, where fertilization takes place. In two South American species, *Eledone massyae* and *Eledone gaucha*, unusual filamentous structures were observed in the animal pole of the oocyte and were entangled greatly with the sperm masses. These structures are extensions of the surrounding layers of the oocytes. The sperm penetrate the filaments forming agglomerates surrounded by a layer of follicular cells. The filaments shorten as the oocyte grows, drawing the enclosed spermatozoa to the ooplasm, in which fertilization occurs. These filaments allow sperm to be stored for long periods and might be analogous to spermathecae in the oviducal gland of Octopodinae.

Key words: Cephalopoda, *Eledone*, fertilization, sperm storage, Octopodidae, Brazil.

INTRODUCTION

Female incirrate octopods can store sperm for a long time after mating. In two of the four Octopodidae subfamilies, the Octopodinae and Bathypolypodinae, internal sperm storage mechanisms and fertilization have been described (Petersen, 1959; Froesch & Marthy, 1975; Wells & Wells, 1977, O'Dor & Malacaster, 1983). Less is known about the Eledoninae and Graneledoninae. In *Eledone cirrhosa*, a time lag between copulation and spawning has been established in aquaria experiments (Mangold et al., 1971) but mechanisms of sperm storage are uncertain (Boyle, 1983).

Eledone massyae Voss, 1964, and *Eledone gaucha* Haimovici, 1988, were described recently and are little known. Haimovici & Andriquetto (1986) and Haimovici (1988) described morphological differences between these species and commented on their apparent geographical coexistence on the southern Brazilian shelf. Some unusual oocyte structures were observed in the ovaries of both species. In this paper these structures are described and evidence is given for their involvement in sperm storage and fertilization.

MATERIAL AND METHODS

Reproductive organs of nine females of *Eledone massyae* and four females of *E.*

gaucha were examined (Table 1). Octopuses were collected with a bottom trawl and fixed in 10% formalin or seawater Bouin solution. Oocytes and oviducal glands were dissected and embedded in paraffin (58°C) according to standard histological techniques (Gabe, 1968). Longitudinal and transverse sections (5 to 7 µm) were stained with Harry's hematoxylin-eosin.

Terms applied to cephalopods and used in this paper:

Spermatangia: Also called sperm sac. Evaginated spermatophores; bladders enclosing the sperm mass.

Spermatophore: Complex sperm package used for transfer of sperm from male to female.

Spermatheca: Seminal receptacle. Pouch in females in which male gametes are stored at mating.

RESULTS

Oocytes

Maturing oocytes remain attached by stalks to the inner epithelium of the ovary (Fig. 1A). Each oocyte is enveloped by three layers: externally, a stratified epithelium of squamous cells; internally, a layer of follicular cells; and between these, a layer of connective tissue

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TABLE I. Females of *Eledone massyae* Voss, 1964, and *Eledone gaucha* Haimovici, 1988, used for histological analyses.

Species	Number	Locality	Date	DML range (mm)	Oocyte length range (mm)	Fixative
<i>E. massyae</i>	5	off Rio Grande do Sul state	Oct. 1988	25.0–65.0	0.7–10.0	Formalin 10%
<i>E. massyae</i>	4	off Rio de Janeiro state	Nov. 1988	64.0–73.3	3.8–6.6	Seawater Bouin
<i>E. gaucha</i>	4	off Rio Grande do Sul state	April 1983	25.0–39.0	0.5–5.4	Formalin 10%
<i>E. gaucha</i>	1	*	Nov. 1983	36.0	5.8	Formalin 10%

with cells of different sizes and shapes, fibroblasts and blood vessels. The follicular cells proliferate and penetrate the ooplasm, forming longitudinal folds that give the oocyte a striped appearance. At final stages of oogenesis these cells secrete the chorion. At the stalked end of the oocyte the chorion becomes drawn out into a stalk (Fig. 2d).

At the animal pole, opposite the stalked end, there is a conical filamentous projection (Fig. 1B). Filament sizes range from twice the length of a 3 mm long oocyte to one-fourth that of the 11 mm long oocytes. Microscopically, the oocyte-surrounding layers are drawn out from the oocyte to form these extensions (Fig. 3D). The external epithelium and the intermediate connective tissue line the filament throughout its length. The follicular cells of the inner layer differentiate and penetrate the filament, filling it as a compact tissue. In the initial stages of maturation (Fig. 3A), follicular cells are dispersed irregularly and are fusiform with elongated nuclei. As maturation advances, these cells become regularly dispersed and cuboidal with large oval nuclei (Fig. 3B). Finally, in advanced stages of maturity, they have smaller nuclei with dense chromatin, which suggests a degeneration of the tissue (Fig. 3C).

Oviducal glands

In the mid-portion of each oviduct is an oviducal gland structurally divided into two concentric glands around the oviduct and separated by a thin sheet of connective tissue (Fig. 3E). Spermatheca are absent (Fig. 4), as in *E. cirrhosa* and *E. moschata* (Froesch & Marthy, 1975). The peripheral gland is formed by groups of concentric cells with basal nuclei and a central lumen; in females close to maturity their cytoplasm is densely packed with reddish grains. The central gland is com-

posed of 22–23 ducts around the oviduct, each lined by an epithelium with ciliated cells having superficial nuclei and glandular cells having basal nuclei. Spermatozoa were not seen to be associated with the oviducts.

Sperm storage and fertilization

In females that had mated, spermatangia and free sperm were seen within the ovaries. As many as seven spermatangia were inside an ovary, often attached to the head of the spermatophoric tunic (Fig. 1D). Free sperm masses occurred around the oocytes and were much entangled with the oocyte apical filaments (Fig. 1C, 1D). Sperm were attached to the filament tips, and apparently penetrated them. Longitudinal sections of apical filaments of the oocyte showed hair-like masses of spermatozoa, regularly dispersed and surrounded by flattened differentiated follicular cells forming dark purple agglomerates along the filament (Fig. 3G, H; Fig. 5). During development, the evolving layers degenerate, thus shortening the filament. The filament-enclosed spermatozoa are thus drawn to the ooplasm, in which fertilization occurs, probably very shortly before spawning (Fig. 2).

Oocytes in females of *Eledone massyae* bearing spermatangia, free sperm and traces of fertilization ranged from 3.0 to 9.0 mm. In *E. gaucha* they ranged from 0.8 to 5.0 mm. In the former, the largest oocyte, although striped, was 12.0 mm; in the latter, it was 7.5 mm (Perez & Haimovici, MS).

DISCUSSION

The potential for storage of sperm is known for octopods of the subfamilies Octopodinae and Bathypolypodinae. The sperm released into the oviducts after the "spermatophoric re-

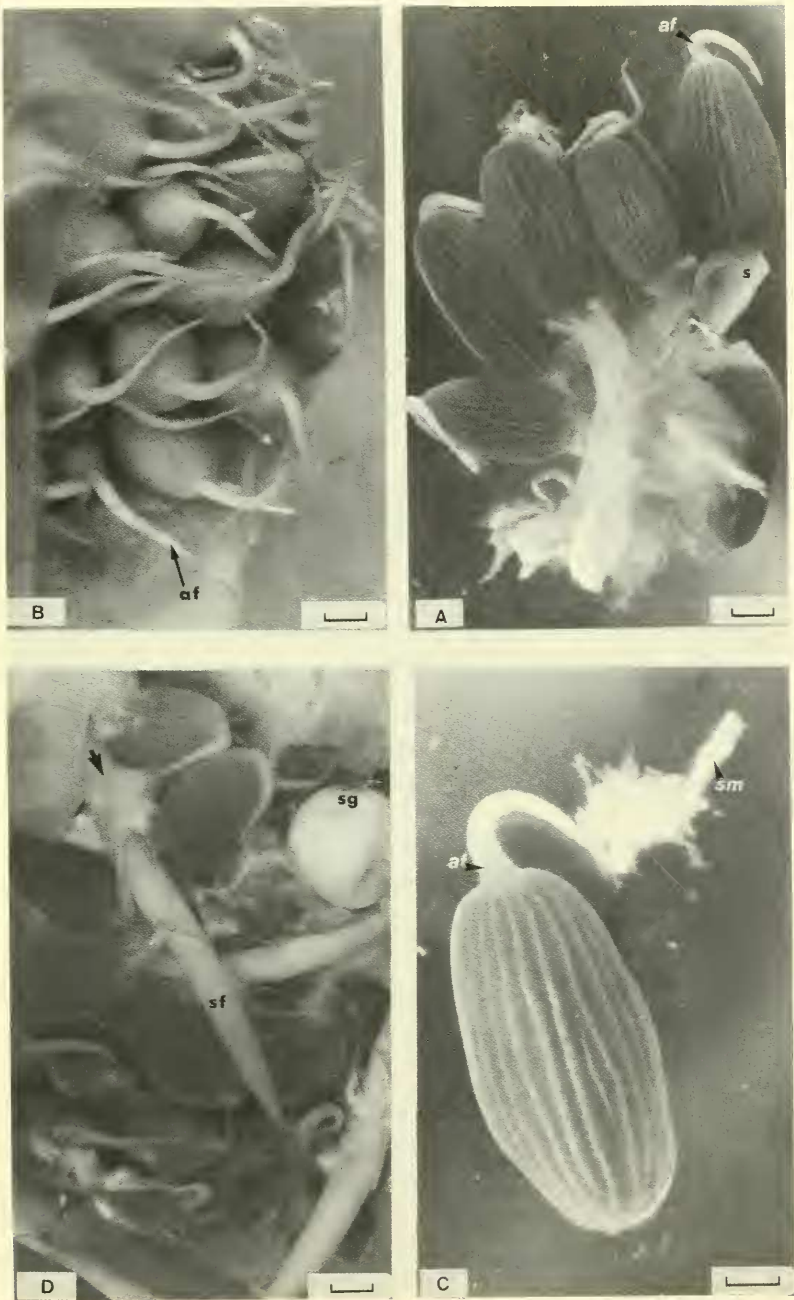


FIG. 1. Photomicrographs of ovary and maturing oocytes of Brazilian *Eledone* species. A. Cluster of maturing oocytes. B. General view of ovary. C. Detail of apical filament attachment to free sperm mass. D. Ovary of mated female. Spermatangia, spermatophore tunics and free sperm mass present. Arrow indicates apical filament attached to sperm mass. Scale bar = 1 mm. af, apical filament; s, stalk; sf, spermatophore tunic; sg, spermatangium; sm, sperm mass.

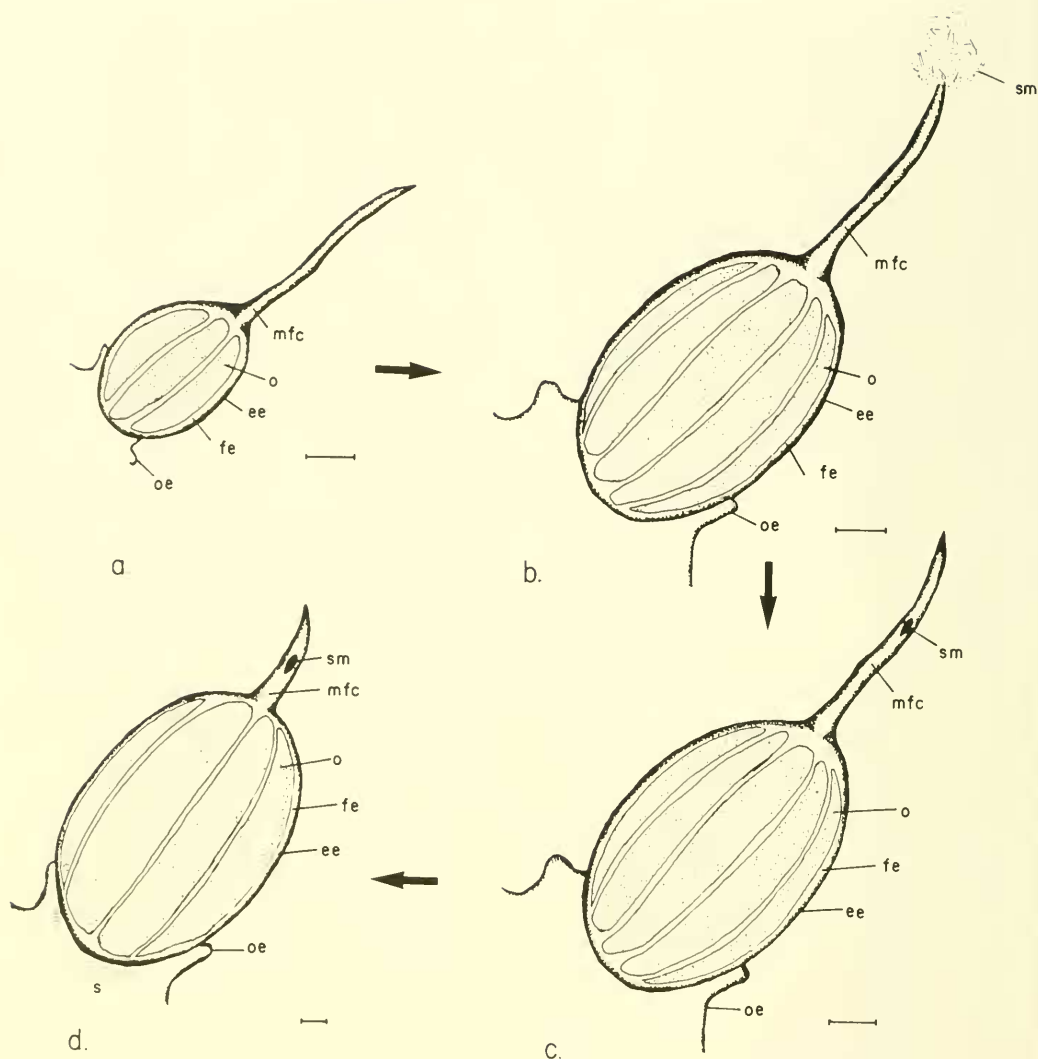


FIG. 2. Schematic diagram of fertilization in *Eledone massyae* and *Eledone gaucha*. a. Oocyte in early stage of maturation. Apical filament is almost twice oocyte length. b. Maturing oocyte. Apical filament as long as oocyte and attached to free sperm mass. c. Maturing oocyte. Sperm mass penetrates filament and is surrounded by modified follicular cells. d. Oocyte in advanced stage of maturation. At this stage, apical filament is very short and sperm mass close to ooplasm. Note position at which it will form chorionic stalk. ee, external epithelium; fe, follicular epithelium; mfc, modified follicular cells; o, ooplasm; oe, ovarian epithelium; sm, sperm mass.

action" (Mann et al., 1970) enter the oviducal glands, where they remain attached to the epithelium of the spermatheca (Froesch & Marthy, 1975). Spermatozoa can be maintained as long as ten months, as observed in *Bathypolypus arcticus* (O'Dor & Malacaster, 1983); thus, mating can occur long before maturation.

Mature eggs are fertilized in the lumina of the oviducal glands just before spawning.

There is evidence of sperm storage in both European and South American species of *Eledone*. Aquaria observations of Mediterranean *E. cirrosa* suggested that sperm might be stored for at least six weeks (Mangold et

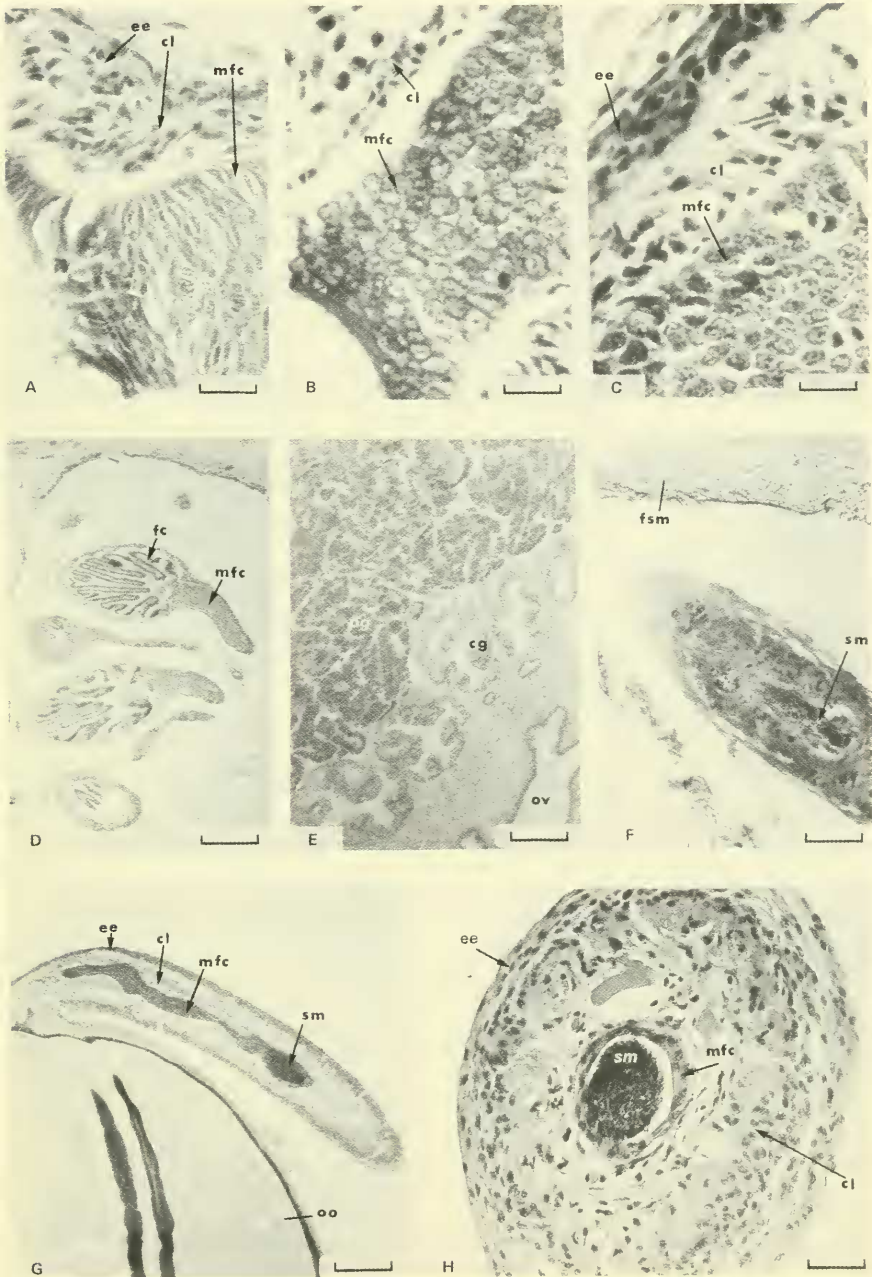


FIG. 3. Photomicrographs of cross-sections of apical filaments, oviducal glands and oocytes of Brazilian *Eledone* species. A-C. Differentiation of follicular cells at base of apical filament, at successive stages of maturation. A, B, scale bar = 20 μm; C, scale bar = 100 μm. D. Oocytes in initial maturation stages showing oocyte surrounding layers forming apical filaments. Scale bar = 300 μm. E. Transverse section of oviducal glands. Scale bar = 300 μm. F. Apical filament tip in contact with free sperm mass. Scale bar = 140 μm. G. Longitudinal sections of apical filament showing surrounding layers and enclosed sperm mass. Scale bar = 300 μm. H. Transverse section of apical filament showing three surrounding layers and central spermatozoa. Scale bar = 70 μm. cg, central gland; cl, connective layer; ee, external epithelium; fc, follicular cells; fsm, free sperm mass; mfc, modified follicular cells; oo, oocyte; ov, oviduct; pg, peripheral gland; sm, sperm mass.

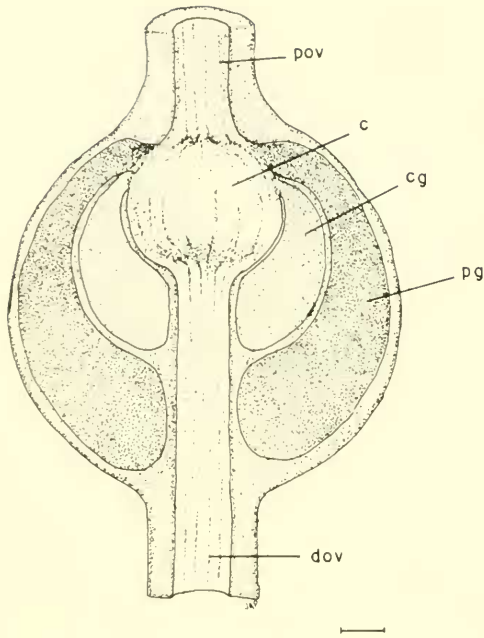


FIG. 4. Schematic diagram of longitudinal cut of oviducal gland of South American *Eledone*. c, central cavity of oviducal gland; cg, central gland; dov, distal oviduct; pg, peripheral gland; pov, proximal oviduct.

al., 1971). A maximal lapse of three months between copulation and spawning was estimated for *E. massyae* (Perez & Haimovici, MS). Maturing females bearing spermatangia within their ovaries were observed in South American species of *Eledone* and Mediterranean *E. cirrosa* (Mangold-Wirz, 1963). In populations of *E. cirrosa* in the North Sea (Boyle & Knobloch, 1983) and *E. moschata* in the Mediterranean (Mangold, 1983) mating was assumed to occur just before spawning because females bearing spermatangia within their ovaries were scarce and nearly mature.

The absence of spermathecae in the oviducal glands seems to be a consistent characteristic of the genus *Eledone* since it was observed in four species. Spermatophores penetrate the oviducts and oviducal glands reaching the ovarian cavity, in which the spermatophoric reaction takes place. Sperm masses occur either freely around the oocytes or enclosed in bladders known as spermatangia (Fort, 1937; Mangold-Wirz, 1963) or sperm sacs (Boyle, 1983; Mangold, 1986). In *Eledone massyae* and *Eledone gaucha*, the apical filaments provide a site for

sperm storage and a fertilization mechanism. Modified follicular cells surrounding the sperm mass inside the filament are supposed to keep spermatozoa viable until oocytes are ripe. Whether the apical-filament mechanism is an adaptation of the entire genus is still unclear. In *E. cirrhosa* and *E. moschata*, sites and timing of fertilization, as well as the means to keep sperm viable, are not known. Early descriptions of the reproductive system of *E. cirrhosa* (Insgrove, 1909; Morales, 1958) do not mention structures similar to the apical filaments. Photographs of ovarian eggs of the same species in Mangold-Wirz (1963: plate II, d, e, f) show delicate expansions at the animal pole of the eggs, although they are quite different from those of the South American species (Fig. 1B). Boyle (pers. comm.) observed white masses of sperm attached to the apical end of each egg. Whether these structures indeed form part of the egg-surrounding layers is not known. Comparisons could not be made with the three remaining but poorly-known described species of the genus, *E. caparti* Adam, 1950, *E. thysanophora* Voss, 1962, and *E. nigra* (Hoyle, 1910) from the West African coast.

In species of *Eledone* that have been studied, as well as in most Octopodinae and Bathypolypodinae, males mature earlier and remain sexually active for a greater part of life than do females. The ability of females to store sperm means that mating can occur long before spawning. Histological study of *E. massyae* and *E. gaucha* shows that at least in the South American eledonids, females can copulate a considerable time before maturation and store sperm until fertilization shortly before spawning. The apical filament and the oviducal glands' spermatheca both allow storage of sperm and are important facets of the reproductive strategy of these octopods.

There are some slight differences between these adaptations, however. In the Octopodinae and Bathypolypodinae, each mature oocyte is fertilized as it descends through the lumen of the oviducal gland (Froesch & Marthy, 1975). Spermatozoa stored in the outer parts of the spermathecae will compete to fertilize the eggs; the last male to copulate with the female will be most likely to sire the offspring. In the South American eledonids the sperm of the first male to copulate are likely to fertilize most of the eggs. This fact could explain the difference in body sizes of the sexes of *E. massyae* at maturity (Perez & Haimovici, MS). If they can mate successfully, young

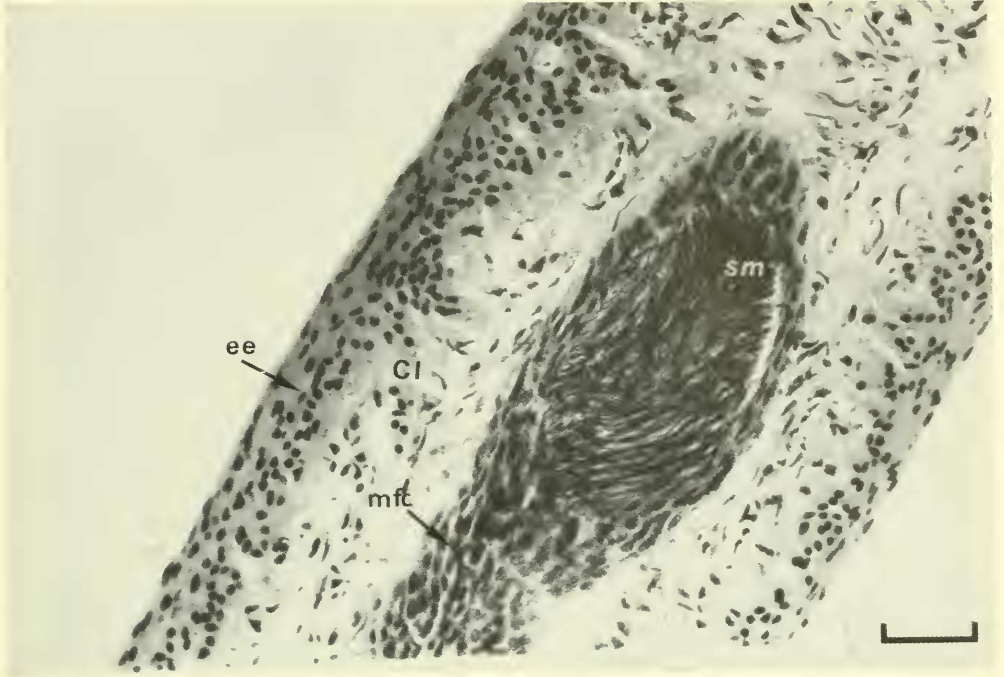


FIG. 5. Longitudinal sections of oocyte apical filament of *Eledone massyae* showing surrounding layers and enclosed sperm mass. cl, connective layer; ee, external epithelium; mfc, modified follicular cells; sm, sperm mass. Scale bar = 40 μ m.

males need not grow so large or live so long as females. Indirectly this difference in size could be particularly advantageous during the spawning and brooding seasons because males would not be in the same areas at the same time with mature females; this separation would not only reduce intraspecific competition for food but also avoid cannibalism on the hatchlings.

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